From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet)	
Applicant's or agent's file reference see form PCT/ISA/220	**FOR FURTHER ACTION** See paragraph 2 below
International application No. PCT/EP2004/007052	International filing date *(day/month/year)* 29.06.2004
Priority date *(day/month/year)* 04.07.2003	
International Patent Classification (IPC) or both national classification and IPC C12Q1/68	
Applicant EPIGENOMICS AG	

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Leber, T Telephone No. +49 89 2399-7195

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-11,26
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-30
Industrial applicability (IA)	Yes:	Claims	1-30
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Basis for the assessment of novelty, inventive step and industrial applicability

1.1 Reference is made to the following document/s/:

D1: PHAM PHUONG ET AL: "Processive AID-catalysed cytosine deamination on single-stranded DNA simulates somatic hypermutation." NATURE (LONDON), vol. 424, no. 6944, 3 July 2003 (2003-07-03), pages 103-107, XP002302398 ISSN: 0028-0836

D2: BRANSTEITTER RONDA ET AL: "Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of Rnase." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 100, no. 7, 1 April 2003 (2003-04-01), pages 4102-4107, XP002302399 ISSN: 0027-8424

D3: EDITED BY S. BECK AND A.OLEK: "The Epigenome" 2003, WILEY-VCH VERLAG GMBH , WEINHEIM, GERMANY , XP008037599

2. Novelty

2.1 Document D1 discloses a method for detecting cytosine methylations in partially single stranded DNA by contacting the DNA to be investigated with an activation-induced cytidine deaminase (AID) converting cytidine into uracil but leaving methylated cytidine unchanged, investigating the deaminated nucleic acid and concluding therefrom on the methylation status of the DNA sequence. D1 shows that AID has a sequence preference for its activity. Thus, the DNA may only be partially be deaminated (D1, whole document). Claims 1-3, 26 lack novelty (Art 33(2) PCT). The same subject-matter is also disclosed in D2 (D2, whole document). Thus, claims 1-3, 26 lack also novelty over D2 (Art 33(2) PCT).

2.2 D2 further discloses that the DNA to be analysed hybridises with an oligomer

whereby a "DNA bubble" is formed resulting in that the nucleotide to be investigated localised in the single stranded part. The said single stranded part may be 1, 3, 4, 5 or 9 nucleotide in length whereby the oligonucleotide has a length of 27 nucleotides. The substrate is present at a concentration of 100nM (D2, Table 1; page 4106, right col.). Thus, also claims 4-11 lack novelty over D2 (Art 33(2) PCT).

3. Inventive step

3.1 Claim 30 differs from the subject-matter disclosed in D1 in that the reagents for deamination are provided in form of a kit.
The technical problem appears to be the provision of the assay reagents in a useful form.
It appears that an inventive step (Art 33(3) PCT) cannot be acknowledged for the solution provided in claim 30, namely the provision of a kit, as it represents a standard procedure for the skilled person to convert a successful laboratory method in to a kit which permits the less experienced to perform a technically demanding technique.

3.2 Claims 24, 25, 27 and 28 refer to the use of the method of claim 1 for, among others, diagnosis of cancer or differentiation of cells. D3 discloses the biological role of cytidine methylation in gene silencing, development, abnormal methylation in cancer cells, ageing etc. (D3, pages 7-15) and reviews methods of DNA methylation analysis. It would therefore appear that no inventive step can be acknowledged for the said claims referring to the obvious combination of a known method of cytidine methylation analysis (see D1 and D2, items 2.1 and 2.2 above) with the disclosure of D3 (Art 33(3) PCT).

3.3 Dependent claims 12-23 and 29 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step, as the said claims refer to features which fall into the conventional modifications introduced into a method of cytidine methylation detection (Art 33(3) PCT).

4. Industrial applicability

4.1 The subject-matter disclosed in the claims 1-30 of the present application appears to be industrially applicable (Art 33(4) PCT).

Re Item VII

Certain defects in the international application

1. The present application does not meet the requirements of Art 5 and Rule 5 PCT as documents D1-D3, which represent relevant prior art, are not referred to therein.